Supporting Information

Kocatürk et al. 10.1073/pnas.1307100110

SI Text

Reagents. Full-length tissue factor (flTF)-specific antibody 10H10, protease activated receptor (PAR)2-, FVII(a)-, and the β1 integrin antibody AIIB2 were described previously (1). p21 antibody, β1 integrin antibody (residues 579–799), β3 integrin-blocking antibody, and HUTS-21 (an antibody that recognizes the active conformation of β1 integrins) were from Millipore. Rabbit monoclonal antibodies against p27 and human alternatively spliced TF (asTF) (RabMab1, custom made) were from Epitomics. p-MAP kinase/MAP kinase antibodies were from Cell Signaling Technologies. The β1 peptide was from Prospec-Tany Technogene. flTF-specific antibody and asTF-specific rabbit polyclonal antibody used for immunohistochemistry were from American Diagnostica [4509 (main text, Fig. 1 legend)] or described previously (2), respectively. Mac3 and Ki67 antibodies were from BD Biosciences. Alexa-488 or Alexa-594-labeled antibodies were from Invitrogen.

Cell Culture, Transfections, and Viral Transductions. All cells were cultured in DMEM (PAA) with 10% serum, 2 mM L-glutamine, penicillin, and streptomycin. FRT sites were introduced to MCF-7 cells using Lipofectamine 2000 (Invitrogen). Cells were subsequently cultured in media containing 100 μg/mL Zeocin, FRT insertion was verified by determining β-Galactosidase expression on Western blot. FRT cell lines (2A3-3 and 2A1-2) were co-transfected with recombinase-encoding pOG44 and pcDNA5-FRT to produce control cells. Similar transfections were carried out with pcDNA5-FRT vector containing cDNA encoding flTF or asTF to produce TF variant-expressing cell lines. The cells undergoing homologous recombination were selected in media containing 150 μg/mL hygromycin. Transductions were carried out with shRNA lentiviral particles prepared from the Mission Library (Sigma-Aldrich). Transduced cells were selected with 2 μg/mL puromycin.

Cell Proliferation and Apoptosis Assays. Cell proliferation was assessed using MTT assays as described previously (3). In some experiments, outcomes of MTT assays were verified using cell counting and DNA analysis. In brief, cells were seeded in 10-cm dishes, lifted, and counted at day 0 and day 3. Proliferation was expressed as an increase in cell number compared with the cell number at day 0. For DNA analysis, cells were lysed in SDS buffer at day 0 and day 3 and DNA contents were measured using Nanodrop. When appropriate, flTF- (10H10, 50 μg/mL), asTF- (RabMab1, 50 μg/mL), β1- and β3 integrin antibodies (50 μg/mL), or a β1 peptide (1 nM) were added. To measure apoptosis, cells were lifted, incubated with Annexin V/propidium iodide (both Sigma-Aldrich) and measured by FACS (LSR2; Becton Dickinson).

Western Blotting. Cells were lysed in sample buffer (Invitrogen). Cell lysates or purified proteins were run on 8–16% gradient gels and transferred to PVDF membranes. Membranes were blocked in 5% nonfat milk powder and incubated with the appropriate primary antibodies, followed by HRP-conjugated secondary antibodies. Protein bands were visualized using Western Lightning ECL (PerkinElmer) and Kodak film (X-Sanatec).

Integrin ELISA. Polysorb 96-well plates (Nunc) were mock-coated (control) or coated with 3 μg/mL sTF or asTF, and blocked with 1% ovalbumin in PBS/Tween-20 (0.02%). Plates were subsequently exposed to the incubation buffer without or with 5 μg/mL recombinant α6β1 (Immunosource), TS2/16 (anti-integrin β1), and peroxidase-conjugated goat-anti-mouse secondary antibody for 60 min at 37 °C. After washing, TMB was added and the reaction was stopped with H_2SO_4.

Microarray Analysis and Validation. Total RNA was isolated, amplified and biotin labeled for hybridization on Illumina HumanHT-12 v4 Expression BeadChips using the Illumina Total Prep-96 protocol. Bioconductor (www.bioconductor.org) was used for the initial microarray analyses (4). The "lumi" library was used for loading and normalizing the Illumina arrays into R and Loess normalization was applied. A gene-filter library was used for removing genes that did not show significant variations after normalization. Finally, the Limma library was used for performing three statistical comparisons: flTF vs. control, asTF vs. control, and asTF vs. flTF. Selected up-regulated or down-regulated genes were chosen, and expression levels were validated by real-time PCR. The primers are listed in Table S2. The gene array data are accessible at Gene Expression Omnibus (accession no. GSE41872).

Soft Agar Growth Assay. Colony formation in soft agar was assayed by plating 10,000 cells in 0.6% agar/DMEM (wt/vol) on top of a 0.75% agar/DMEM layer in 35 mm dishes. Plates were incubated at 37 °C and 5% CO_2 for 14 d. Colonies were visualized using an inverted microscope (Leica DMIL) and Leica DFC295 camera. Cumulative colony size (area covered) and colony numbers were determined using ImageJ software.

1. Versteeg HH, et al. (2008) Inhibition of tissue factor signaling suppresses tumor growth. *Blood* 111(1):190–199.
2. Srinivasan R, et al. (2011) Splice variants of tissue factor promote monocyte–endothelial interactions by triggering the expression of cell adhesion molecules via integrin-mediated signaling. *J Thromb Haemost* 9(10):2087–2096.
3. Versteeg HH, Evertzen MW, van Deventer SJ, Peppelenbosch MP (2000) The role of phosphatidylinositide-3-kinase in basal mitogen-activated protein kinase activity and cell survival. *FEBS Lett* 465(1):69–73.
4. Gentleman RC, et al. (2004) Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biol* 5(10):R80.

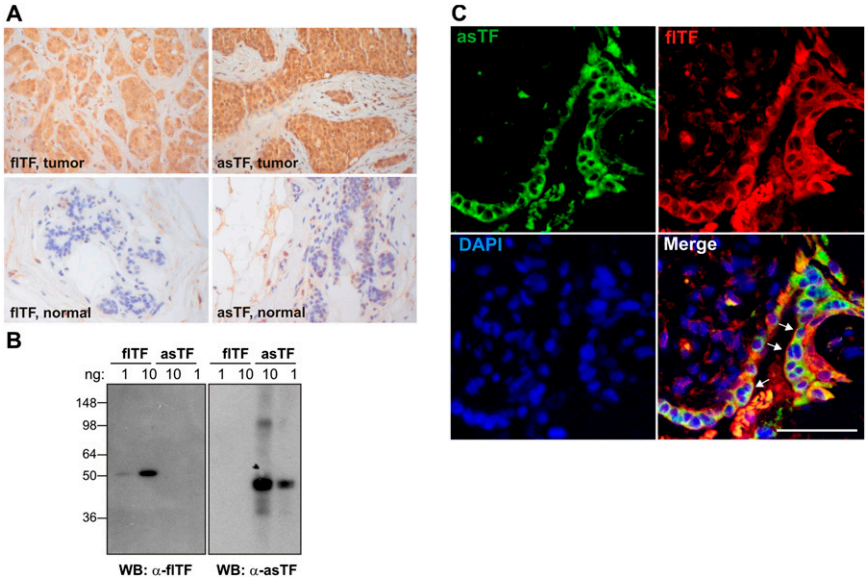

Fig. S1. asTF and fITF are overexpressed in human breast cancer specimens. (*A*) Representative photographs of tissue microarray punches of human breast cancer specimens (*Upper*) and matched normal mammary tissue (*Lower*) immunohistochemically stained using an fITF-specific (*Left*) or an asTF-specific antibody (*Right*). Brown color indicates positive staining. (Magnification: 20×.) (*B*) Specificity of the anti-fITF and anti-asTF antibodies, tested on Western blot. The indicated amounts of purified fITF and asTF proteins were loaded on gel. Note the asTF dimers migrating at 98 kDa. (*C*) Representative confocal image of a breast cancer specimen double-stained with the antibodies in *A*. Note the intracellular staining of asTF (green) and the fITF staining at the cell periphery (red). Examples of peripheral staining are indicated by arrows. (Scalebar: 100 micron.)

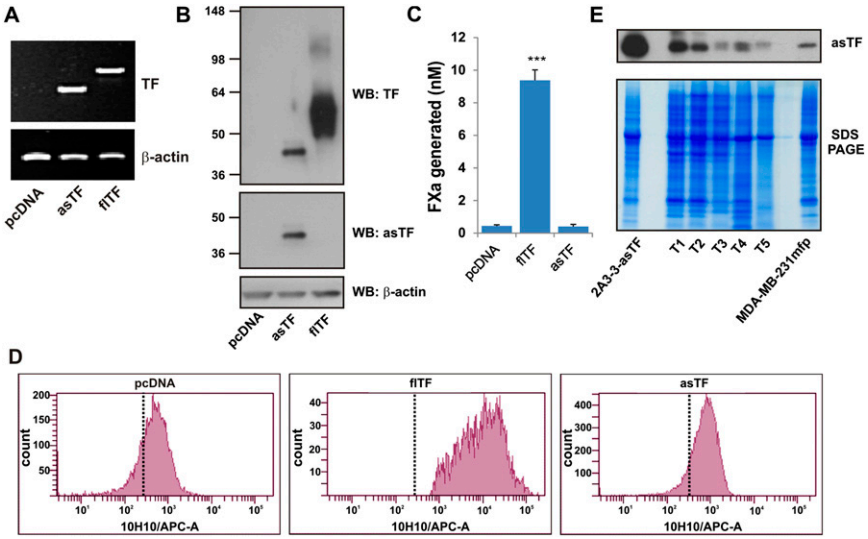

Fig. S2. Characterization of TF isoform-expressing 2A3-3 cells. (*A*) Control vector (pcDNA), fITF or asTF cDNA were stably integrated into the FRT site of 2A3-3 cells and TF isoform expression was verified by PCR using TF-specific primers. Primers against β-actin were used as a control. (*B*) Expression of fITF and asTF in 2A3-3 cells assessed by Western blot. Antibodies specific for total TF and asTF (monoclonal rabbit antibody RabMab1) were used. (*C*) TF activity of 2A3-3 control cells and cells expressing fITF or asTF was measured after addition of 10 nM FVIIa and 100 nM FX in HBS. FXa generation was determined after 30 min using a Spectrozyme FXa kinetic assay. (*D*) FACS analysis of 2A3-3 control cells and cells expressing fITF or asTF. Cells were labeled in suspension with 1 μg/mL 10H10 and APC-labeled rabbit anti-mouse secondary antibody. Labeled cells were analyzed on a Becton Dickinson LSR II. Dashed lines indicate mean fluorescence intensity of the same cells, labeled with IgG control. (*E*) Protein (50 μg) from lysates prepared from 2A3-3-asTF, MDA-MB-231mfp cells and five tumor specimens with high asTF expression (as determined in the tissue array analysis) were subjected to Western blotting using anti-asTF RabMab1 as the primary antibody. The same samples were also loaded on SDS/PAGE and stained using Coomassie to verify equal loading. ***$P = 0.001$.

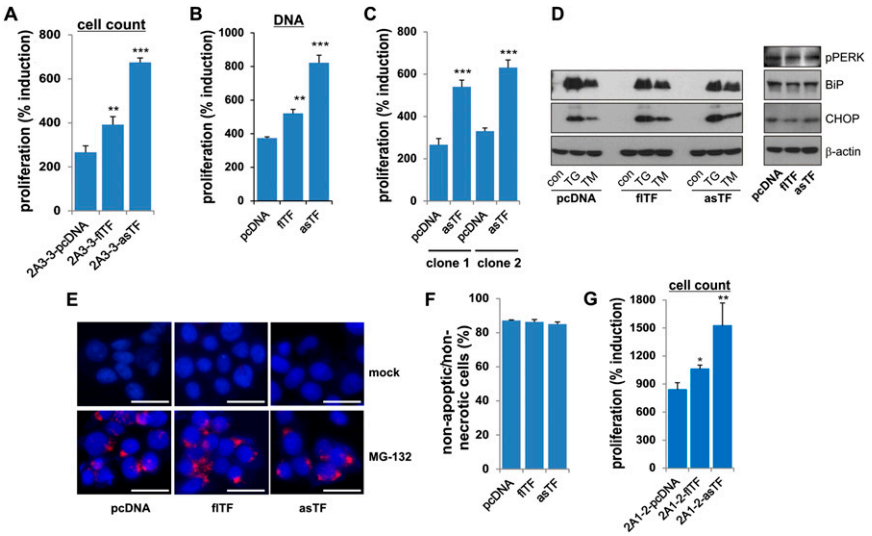

Fig. S3. Proliferation of asTF-expressing 2A3-3 cells. (*A*) Proliferation of 2A3-3 cells expressing TF variants was determined using cell counts. (*B*) Proliferation of 2A3-3 cells expressing TF variants was determined using DNA contents of cellular lysates at day 0 and day 3. Proliferation was expressed as percent increase compared with day 0. (*C*) Enhanced proliferation of an independently generated second asTF clone was determined using MTT assays. (*D*) Activation of the unfolded protein response (UPR), as determined by Western blotting. The indicated cell lines were screened for up-regulation of UPR-responsive proteins CCAAT/-enhancer-binding *protein* homologous *protein* (CHOP) and binding protein (BiP), and phosphorylation of *protein* kinase RNA-like endoplasmic reticulum kinase (PERK). β-Actin was used as a loading control. (*Left*) A short exposure of CHOP and BiP levels in these cells, using established UPR activators [2 μM Thapsigargin (TG) and 10 μg/mL Tunicamycin (TM)] as positive controls. (*Right*) Basal levels (long exposure) of CHOP and BiP, and PERK phosphorylation in these cells. (*E*) Presence of cellular protein aggregates was determined using the ProteoStat protein aggregation kit (Enzo Life Sciences). The proteasome inhibitor MG-132 was used as an inducer of protein aggregation (positive control). Images were acquired with a conventional fluorescence microscope (Leica DMI6000B). (Scale bars, 20 μm.) (*F*) Apoptosis was assessed using Annexin V-FITC staining and subsequent FACS analysis. (*G*) A cell line (2A1-2) containing an FRT site at a transcriptionally less active region was equipped with an empty vector, flTF or asTF cDNA by homologous recombination, and proliferation was monitored using cell counting. $*P < 0.05$, $**P = 0.01$, and $***P = 0.001$.

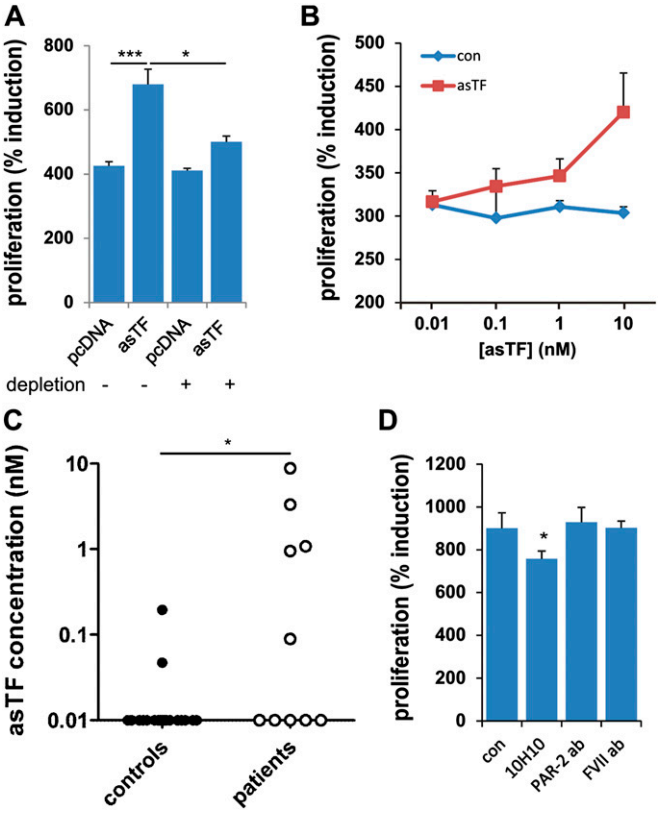

Fig. S4. Proliferation of 2A3-3-asTF cells is dependent on asTF secretion. (*A*) Conditioned medium of 2A3-3-asTF cells was left untreated or asTF-depleted using an asTF-specific antibody RabMab1. 2A3-3-pcDNA cells were cultured in these medium preparations, and proliferation was assessed 3 d later using MTT assay. (*B*) Effects of indicated amounts of recombinant asTF on 2A3-3-pcDNA cell proliferation using MTT. The experiment was controlled (con) by adding the identical corresponding amounts of the vehicle. (*C*) asTF concentrations were determined in plasma from 20 healthy control subjects and 10 metastatic breast cancer patients using an asTF-specific ELISA. (*D*) flTF cells were incubated with 50 μg/mL 10H10, PAR-2 blocking antibody, or FVII-blocking antibody. Increases in cell number were determined on day 4, using MTT assay. $*P < 0.05$ and $***P = 0.001$.

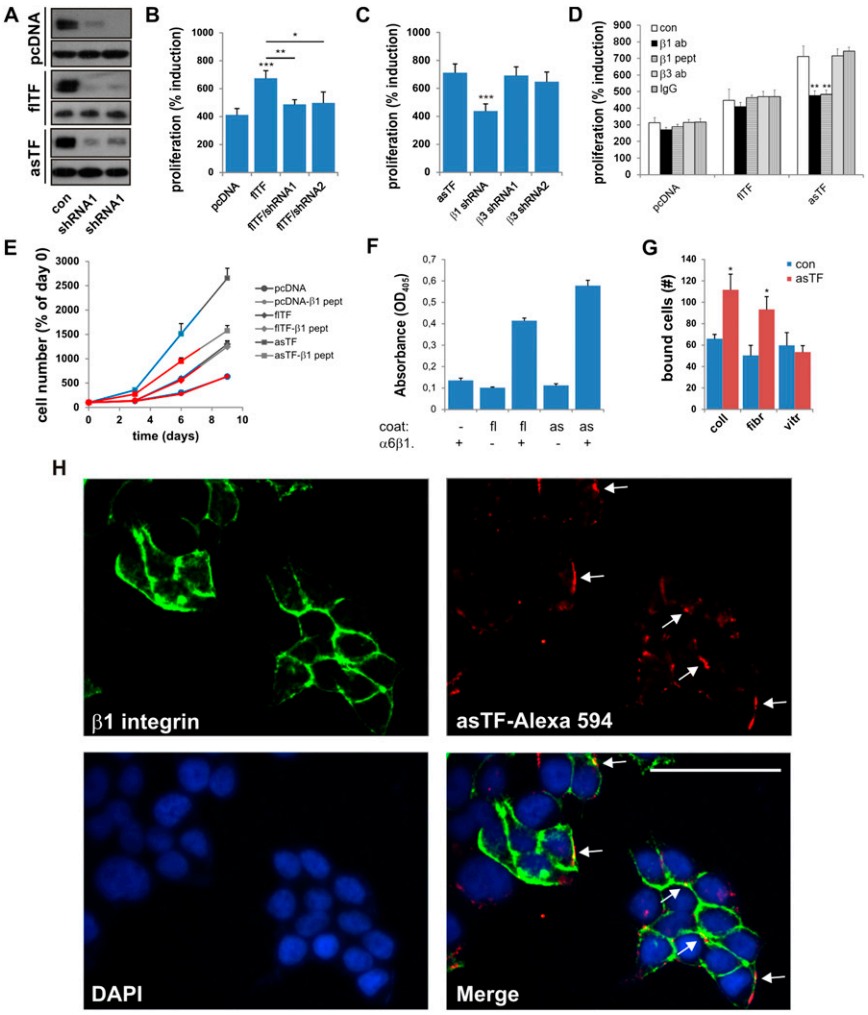

Fig. S5. Effect of integrin blockade on flTF- or asTF-expressing 2A3-3 proliferation. (*A*) 2A3-3-pcDNA, -flTF, and -asTF cells were transduced with β1 integrin shRNA lentiviral particles. The Western blot shows β1 integrin expression levels after lentiviral transduction, β-actin is used as a loading control. (*B*) Proliferation of β1-silenced 2A3-3-flTF cells was assessed after 3 d using MTT assay and compared with that of control cells (pcDNA). (*C*) Cells were transduced with β1 integrin-specific shRNA particles or β3 integrin-specific shRNA particles as a control. Cell proliferation was determined by means of MTT assay. (*D*) 2A3-3 cells were incubated with antibodies against β1 integrins (residues 579–799), β3 integrins, or a peptide representing integrin β1 residues 579–799. (*E*) Proliferation was assessed after the days indicated as described. 2A3-3-pcDNA cells (circles), -flTF cells (diamonds), or -asTF cells (squares) were incubated with a β1 peptide resembling the β1 integrin region between residues 579–799 (1 nM). Proliferation was followed as in *A*. (*F*) The 96-wells plates were coated with asTF. Truncated flTF (sTF) coating was included as a positive control, as flTF was previously shown to bind to α6β1 integrins. Subsequently, the plates were incubated with the indicated recombinant integrin dimers, and bound integrin was detected using β1 antibody TS2/16 HRP-conjugated anti-mouse secondary antibody and colorimetric substrate (TMB). (*G*) 2A3-3-pcDNA cells were preincubated with 10 nM recombinant asTF and seeded on collagen I, fibronectin, or vitronectin precoated dishes (all 10 μg/mL) and left to adhere for 60 min in Hepes-Tyrode buffer. After washing, the remaining cells were counted. (*H*) pcDNA cells were grown on coverslips and incubated with 10 nM recombinant asTF conjugated to Alexa 594 for 20 min at ambient temperature. After fixation, cells were labeled with β1 antibody AIIB2 (green). Arrows indicate areas of extensive colocalization. (Scale bar, 50 μm.) $*P < 0.05$, $**P = 0.01$, and $***P = 0.001$.

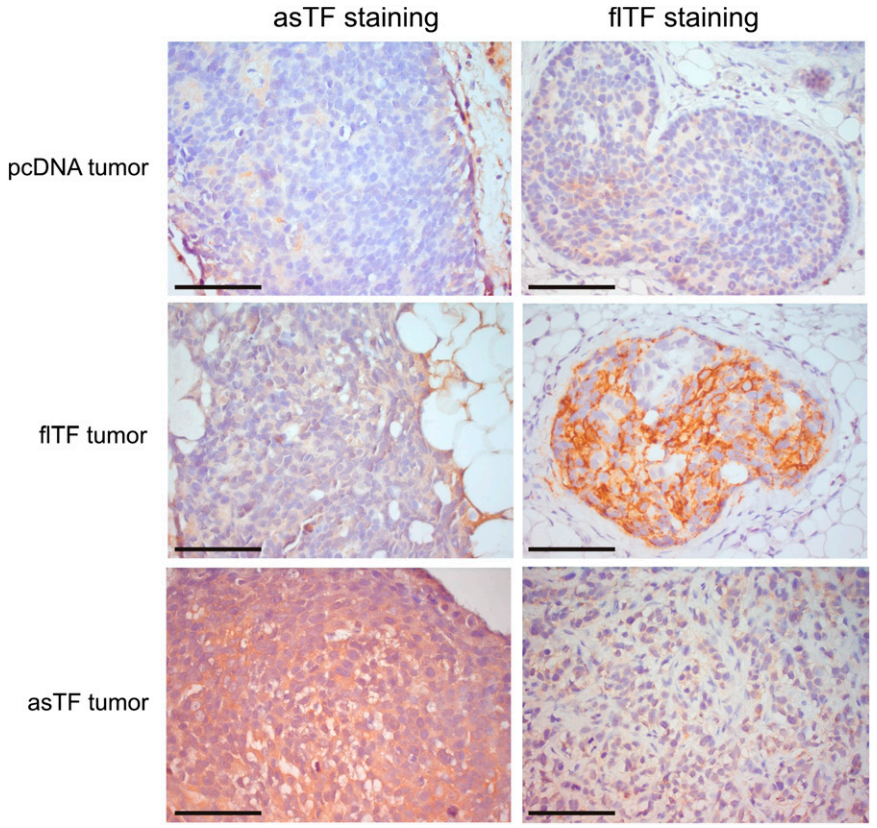

Fig. S6. Morphology and TF variant staining of 2A3-3 tumors. 2A3-3-pcDNA, -flTF or -asTF-derived tumors were extracted, fixed and stained using specific antibodies for asTF (*Left*) or flTF (*Right*). Sections were counterstained with hematoxylin. Note differences in flTF and asTF staining patterns and tumor morphology of pcDNA and flTF tumors versus asTF tumors. (Scale bars, 100 μm.)

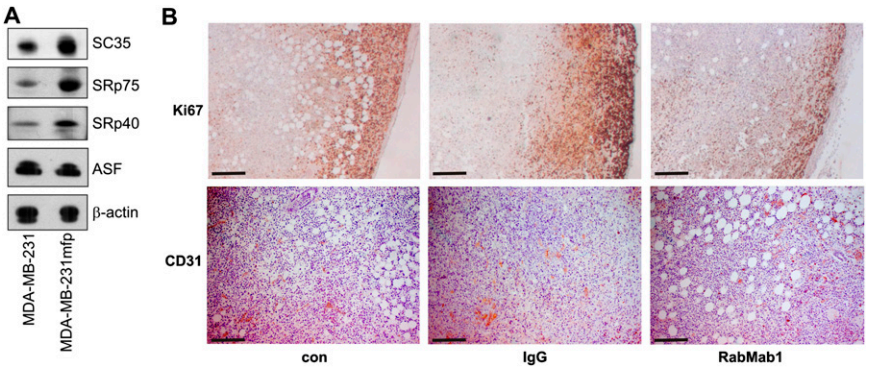

Fig. S7. Characteristics of MDA-MB-231-mfp tumor cells and MDA-MB-231-mfp–derived tumors. (*A*) Expression levels of SC35, SRp75, SRp40, and ASF/SF2 were determined on Western blot. β-Actin served as loading control. (*B*) MDA-MB-231-mfp cells were coinjected with buffer control (con), 100 μg IgG control antibody or 100 μg RabMab1. Tumors were extracted, fixed in formalin, and stained for Ki67 (Magnification: 5×.) or CD31 (Magnification: 10×.). Notice the presence of mammary fat cells in the tumor specimens (white). (Scale bars, 200 μm.)

Table S1. List of genes included in the heatmap in Fig. 2*A*

Gene	asTF vs. control	*P* value	flTF vs. control	*P* value
Cytoskeleton regulation				
LCP1	3.742041408	4.82E-07	1.597922314	0.004017
SCIN	2.108646599	7.30E-09	1.329457586	0.000118
TUBB2B	1.928582645	0.000285	1.477838294	0.010475
CORO1A	1.868572756	8.27E-06	2.14044709	1.17E-06
UBE2L6	1.780560434	0.000203	2.210948723	1.14E-05
RDX	1.749752453	1.33E-06	−1.313590672	0.000873
HAX1	1.7014436	1.56E-05	2.036833659	8.66E-07
LOC606724	1.635658577	4.22E-05	2.067936846	9.50E-07
STMN3	1.470501982	0.001837	1.852729618	3.99E-05
TWF1	1.390079424	1.38E-05	1.047946252	0.326043
RHOD	1.398217432	0.001541	−1.418470373	0.001135
Integrin and migration				
BGN	1.859405347	0.000335	−1.049253889	0.703295
FERMT2	1.857779519	2.67E-06	1.193333563	0.032271
SMAGP	1.530149351	7.72E-05	1.574769438	4.29E-05
TRIP6	1.433852054	8.72E-06	1.447503733	6.78E-06
ID1	1.429662141	0.024691	2.92285299	6.89E-06
TM4SF1	1.422992101	0.041298	−1.623238739	0.008735
ZFYVE21	1.408507583	1.50E-06	1.303102493	1.87E-05
ITGAE	1.370217825	1.44E-05	−1.093804744	0.064612
RAPGEF2	1.341491552	3.69E-06	1.019520069	0.597044
Angiogenesis inducer				
CTSH	2.078278757	1.30E-06	1.227613867	0.025359
PTGES	1.804505885	1.98E-05	−1.16678851	0.095746
METAP1	1.451736383	4.43E-06	1.279896922	0.000197
HIF1A	1.442084355	0.000576	−1.069079685	0.407952
NCL	1.42837763	0.000151	−1.02074889	0.755335
PLA2G3	1.34121158	8.50E-06	1.361032506	5.28E-06
Cell cycle				
CCNA1	3.444212992	1.03E-09	1.311815883	0.00223
TEAD2	1.999153596	0.001922	2.719114401	0.000105
CCNA2	1.689827846	1.02E-07	1.351804407	2.68E-05
ANAPC10	1.684016033	9.30E-08	1.174051955	0.003684
MAD2L1	1.584491419	1.23E-05	1.192226934	0.017
CBL	1.506003722	0.000116	−1.00792761	0.914163
CDC5L	1.45321681	0.001637	1.130303557	0.205681
ID3	1.444140914	0.021992	1.864606082	0.000831
ID1	1.429662141	0.024691	2.92285299	6.89E-06
PIR	1.376465624	2.23E-05	1.146793418	0.012949
LIN54	1.373650529	0.000956	1.261968371	0.007639
MNAT1	1.37187435	0.002633	−1.05517336	0.528861
LCMT1	1.368999315	0.000314	1.35855059	0.000383
MRS2	1.360460762	1.16E-05	1.079358084	0.094399
NUP153	1.351409693	0.000212	1.077304377	0.213649
GMNN	1.344105524	0.008987	1.090079751	0.37861
Tumor cell proliferation				
H19	3.489019144	2.00E-12	2.954286767	1.01E-11
MDK	2.454063596	2.31E-05	1.387160376	0.029261
GAL	2.037698068	0.000246	1.512087264	0.010815
PCSK1N	1.862575221	1.26E-06	1.567716533	2.98E-05
MND1	1.860190359	6.09E-07	1.438271735	0.000105
ODC1	1.834345353	1.52E-06	1.81512049	1.82E-06
SLC16A3	1.761121624	0.000333	1.313471999	0.032633
TIMP1	1.760619541	0.000204	1.180564302	0.143626
TBX2	1.658101848	0.000721	−1.381987092	0.013261
PHF19	1.647718256	3.45E-05	1.488760883	0.000253
MAPK13	1.536585166	0.000472	2.035143814	5.15E-06
FABP5	1.532500067	1.20E-07	1.384337539	2.01E-06
EIF4E	1.547804854	0.001	1.020291222	0.843895
PFKM	1.481369052	0.000113	1.731273689	5.05E-06
HOXB7	1.463554404	4.50E-05	1.147175144	0.041784
FGFR3	1.462154414	0.000446	1.341156069	0.002959

Table S1. Cont.

Gene	asTF vs. control	*P* value	fITF vs. control	*P* value
MARCKS	1.423358397	0.002797	−1.297591069	0.016804
PRR5	1.422956559	0.000163	1.159269894	0.041068
RAP1GDS1	1.417514749	4.30E-06	1.186787763	0.00195
PPIL1	1.416748859	0.00359	1.195002505	0.087845
PFKP	1.409792362	1.42E-05	1.502888233	2.68E-06
ATOX1	1.405348878	0.000415	2.200283203	1.38E-07
YWHAG	1.400002412	0.000612	1.006903933	0.925548
PLOD3	1.386953228	0.002265	1.22269681	0.034265
PBK	1.362937947	0.00072	1.537087528	4.54E-05
RAP1GDS1	1.347424073	2.88E-06	1.060408762	0.1227
FDFT1	1.344708736	0.134579	1.046267955	0.810124
CALM1	1.341930602	0.000384	1.342218442	0.000381
Invasion				
FAM5C	2.092361538	7.64E-09	1.408929672	2.08E-05
LMO4	1.539098114	1.30E-05	1.395712469	0.000135
ACSL4	1.475175376	1.28E-07	1.333133311	2.87E-06
HMGN5	1.451825346	2.79E-05	1.262108012	0.001409
FGFR4	1.418287964	9.36E-05	1.629394916	4.06E-06
MMP9	1.383610445	1.05E-05	1.308708358	6.07E-05
CUX1	1.383282194	0.00048	1.223505839	0.011696
EGR3	1.354850524	0.002475	1.069302011	0.412034
PTTG1	1.341683457	0.000607	1.656079806	4.92E-06
SEMA3E	1.332027901	0.243818	−1.383155273	0.190906
SMAGP	1.330776688	0.00024	1.415029063	4.39E-05
mRNA splicing				
HNRNPH1	1.928871989	0.000228	1.238105925	0.1129
SNRPC	1.82563735	3.20E-06	1.445397593	0.000289
HNRPDL	1.572832064	9.64E-05	1.353544414	0.002292
HNRNPH3	1.509978879	3.07E-05	1.309311687	0.001083
HNRNPU	1.508793814	0.026946	−1.161521139	0.374949
LSM6	1.485474582	0.003655	1.017512615	0.875937
SRPK1	1.437383427	9.62E-05	1.053853601	0.415897
SNRPC	1.42544196	0.000444	1.353365203	0.001476
PRCC	1.361638994	0.001173	1.226295917	0.015717
THOC5	−1.331761098	0.000936	−1.01730332	0.796002
KHSRP	−1.357140125	0.002549	1.16120239	0.085754
SNRPD3	−1.391235152	2.91E-05	−1.149889184	0.016124
LSM14A	−1.412268479	3.25E-05	−1.276059742	0.000633
TTF2	−1.423558516	0.000219	−1.07828293	0.280668
SRPK2	−1.899174927	1.74E-05	−1.800406495	3.90E-05
Cell survival				
MSLN	1.759854431	5.95E-07	1.142949637	0.038443
GPX3	1.73571357	1.79E-05	1.252757581	0.010156
GPX8	1.644675123	3.80E-07	1.352780874	5.08E-05
UBE2D3	1.52981016	5.55E-05	1.438741757	0.000216
LAMTOR3	1.520041452	8.40E-08	1.178291252	0.000614
SIVA1	1.403788792	0.000222	1.602042598	1.15E-05
BCAS2	1.365068982	0.066403	−1.27417534	0.141433
RBM38	1.362618327	0.005194	1.098192724	0.318759
HMGA1	1.35714425	0.088863	1.393404303	0.067435
WBSCR22	1.342446449	0.000828	1.465913378	9.70E-05
IER3	1.333800324	0.082352	1.506736772	0.019669
Tumor suppressor				
MB	−1.33064222	0.00093	−1.446513458	0.000116
CDH1	−1.332727303	0.00057	−1.202639385	0.011018
STAT1	−1.339818537	5.97E-06	−1.400991988	1.45E-06
PLK2	−1.367464292	4.18E-05	−1.37124953	3.86E-05
CCDC6	−1.370125987	4.79E-05	−1.254761305	0.000751
LRRC26	−1.372949147	7.11E-05	1.170114234	0.011712
ENO1	−1.375689186	3.63E-05	−1.074586971	0.169045
PRDM4	−1.378877472	0.001111	−1.045399441	0.562209
RND3	−1.393657887	0.001223	−1.022880967	0.776587

Table S1. Cont.

Gene	asTF vs. control	*P* value	fITF vs. control	*P* value
KLF6	−1.411761734	9.31E-05	−1.331647695	0.000442
PPP1R7	−1.423655222	4.41E-06	−1.39691304	7.57E-06
SSBP2	−1.448869666	7.34E-07	−1.110176831	0.018871
PTPRK	−1.500527266	9.46E-06	−1.954301708	5.54E-08
RPRM	−1.509953249	1.68E-05	−1.921471447	1.62E-07
LZTR1	−1.517064516	0.002917	−1.191377647	0.140816
PCDH19	−1.524311442	1.20E-05	−1.593325281	4.53E-06
DNAJB6	−1.556756399	1.40E-05	−1.601836929	7.66E-06
RAP1GAP	−1.571112608	0.002977	1.168892538	0.220224
TFF1	−1.584119506	0.000215	1.026526455	0.767586
TSC22D1	−1.616857744	0.00075	−1.879048289	7.87E-05
RHOB	−1.707273726	2.64E-05	−4.267101563	7.97E-10
FHL1	−1.992656625	2.02E-05	−2.11291229	9.29E-06
CDH18	−2.044637464	0.000141	−1.944705571	0.000261
MT1F	−2.517448528	2.52E-05	−2.732116595	1.13E-05
Cell cycle/proliferation inhibitors				
TSPAN13	−1.341011207	0.001989	−1.162470215	0.064723
FBXO4	−1.,343238268	0.00044	−1.190087898	0.014609
E2F4	−1.348242117	0.000776	−1.189584577	0.022659
TP53INP1	−1.348390493	0.001801	−1.625258944	3.36E-05
HDAC1	−1.35523136	7.02E-05	−1.018092077	0.727663
RBL2	−1.411972962	0.001521	−1.368100279	0.002956
CREG1	−1.437544657	1.17E-05	−1.547601463	1.89E-06
RTKN	−1.455857165	0.019402	1.027848036	0.84597
PRKCD	−1.465776602	0.002264	−1.07726449	0.462124
MXD4	−1.469172735	0.007975	−1.077834212	0.544433
DUSP5	−1.492568657	0.027005	−1.050338727	0.761506
STC2	−1.514766507	0.000614	−1.122918866	0.217937
ADIPOR1	−1.591749322	6.85E-06	−1.336800406	0.000459
NR2F2	−1.620623423	2.15E-05	−2.297733652	8.82E-08
SOCS2	−1.63864273	0.007059	−2.367201364	0.000117
LRIG1	−1.970185246	2.49E-05	−1.437044657	0.006081
BTG1	−2.021573027	2.22E-05	−2.48332801	1.82E-06
EFEMP1	−2.504632082	3.96E-08	−2.744676259	1.42E-08
Apoptosis				
LDOC1	−1.35901737	0.105565	−2.417070141	0.000326
TRIB3	−1.371297096	0.00303	−1.134207618	0.16238
PHLDA3	−1.390723569	0.000583	−1.608557746	2.50E-05
FASTK	−1.414327129	0.000663	−1.007112367	0.926319
TSC22D1	−1.414522749	9.57E-05	−1.781944147	7.04E-07
RPS27L	−1.452380564	1.72E-05	−1.752284775	2.92E-07
IFIT1	−1.456319273	0.023422	−1.568748289	0.009149
PPM1F	−1.461355694	0.001109	−1.099442341	0.302576
BCL2L13	−1.636184556	8.21E-06	−1.321207561	0.00113
BHLHE40	−1.636991122	4.07E-06	−1.033776998	0.591939
CERS6	−1.648999442	6.53E-06	−1.210013214	0.012203
STK25	−1.788496464	5.61E-08	−1.339253653	5.48E-05
DRAM1	−1.857101883	4.56E-05	−2.125307827	7.10E-06
SLC39A6	−2.334192998	2.31E-06	−2.321008519	2.47E-06
Migration/invasion/angiogenesis inhibitors				
FOXA1	−1.356460032	0.001706	−1.034143346	0.662339
CITED4	−1.354405823	0.001696	−1.909148902	2.25E-06
EPHA4	−1.400971068	1.24E-06	−1.455333931	4.13E-07
CITED2	−1.657840051	8.46E-07	−1.637358522	1.09E-06
TNS3	−1.911609586	4.48E-07	−2.129502378	2.17E-06

Table S2. Primer pairs used in SYBR-Green based real-time PCR

Gene	Forward primer	Reverse primer
LCP1	TGCCGGCAGTTTGTCACA	GGCAATAAAAGCCAAGTTCAACTT
FERMT2	AAAACAATGACCCCCACTTATGA	GTCACCAAACCAAGCAGAAGTTG
CCNA1	CCATCGACCTCAGCAAGCA	TGGCTCCATGAGGGACACA
MDK	GGTGCCCTGCAACTGGAA	ACGCACCCCAGTTCTCAAAC
FAM5c	CAGCACCCTTCGGAGACTTC	CCAGTCCGTGTTTCTGTTACCTT
MSLN	TGGACTTGGCCACGTTCAT	TGCACCTCAGCCACAGTCA
CDH18	AAACTTCACTCTGAAGGACAATGAAG	CCCATCTCTCTCGCATGCA
EFEMP1	CCAACCCTTCCCACCGTAT	TGTCTTGGCACACGTTGTGTT
DRAM1	AACTTGGTGTCTTTAGTGCTTGGA	ACTCCTGAAAATTGGCGACAA

Primer sequences are from 5′ to 3′. *LCP1*, lymphocyte cytosolic protein 1; *FERMT2*, fermitin family homologue 2; *MDK*, midkine; *MSLN*, mesothelin; *CDH18*: cadherin 18; *EFEMP1*, EGF containing fibulin-like extracellular matrix protein 1; *DRAM1*, DNA-damage regulated autophagy modulator 1; *FAM5c*, family with sequence similarity 5, member C.